



2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

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Assessing Fermentation Quality of Grain Sorghum for Fuel Ethanol Production Using Rapid Visco Analyzer

Renyong Zhao, Graduate Student

Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506

Scott Bean, Research Chemist

USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502

Donghai Wang, Associate Professor

Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506.

Xiaorong Wu, Research Associate

Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506.

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Abstract. *The Rapid Visco Analyzer (RVA) was used to characterize the pasting properties of 68 sorghum grains with a standard 23-min temperature profile. The results showed a strong linear relationship between ethanol yield and final viscosity, as well as setback. Ethanol yield increased as final viscosity decreased. A modified RVA procedure (10 min) with an application of α -amylase was developed to simulate the liquefaction step in dry-grind ethanol production. There was a remarkable difference in mashing property among the sorghum samples with the normal dosage of α -amylase. The sorghum samples, which were difficult to liquefy in the mashing step, had much higher peak viscosities than the samples that were easily liquefied. The results also showed that the relationship between conversion efficiency and mashing property was significant. Tannins were shown to cause high mash viscosities. The modified RVA procedure is applicable not only for characterization of mashing properties, but also for optimization of α -amylase doses for starch liquefaction.*

Keywords. Sorghum, RVA, pasting, amylase, mashing, ethanol, viscosity, tannin

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Introduction

Sorghum is a drought-resistant, low-input cereal grain, and interest in using it for bio-industrial applications is growing in the U.S. (Farrell et al., 2006). Researchers and ethanol producers have shown that grain sorghum is a reasonable feedstock (e.g., technically acceptable, fits the infrastructure, and can be economically viable) for ethanol and could make a larger contribution to the nation's fuel ethanol requirements.

In a conventional, dry-grind ethanol process, sorghum is ground and mixed with water to form mash, which is cooked, liquefied, saccharified and fermented to produce ethanol. Mash used in industry for the production of fuel ethanol usually have a dissolved content in the range of 20-24 g /100 mL of mash, and normally a grain-to-water ratio of 1:3 is used (Thomas et al., 1995). More recently, fuel alcohol plants have run at alcohol levels as high as 19-20% by volume with the average being nearer to 16-17% (Kelsall and Lyons, 2003). Due to the high solids in mash, the viscosity is extremely high during starch gelatinization. In dry-grind processing, thermostable α -amylase enzymes are added as thinning agents to reduce viscosity and partially hydrolyze starch during cooking. Lower mash viscosities improve the heat transfer efficiency in the heat exchangers and allow the plant to process higher levels of dry solids, which gives a significant energy reduction in heating of the mash and cooling of the cooked liquefact prior to fermentation. In addition to high energy consumption, a high viscosity also may result in incomplete starch gelatinization and low ethanol yield. Therefore, the viscosity of cooked mash could be used as a quality factor for optimizing the solids content in the mash, stirring system, and amylase levels.

RVA has been used to study starch pasting properties through the classic heat-hold-cool profile (Wrigley et al., 1996). Compared with wheat and corn, fewer studies have reported on using RVA to study grain sorghum and its associated products. RVA has been mostly used to investigate the pasting properties of isolated sorghum starches or starches in raw, dehulled, decorticated, flaked, and malted sorghum grains, with solid levels ranging from 8.6 to 14.0% (w/w) in the slurries (Agu et al., 2006; Beta and Corke, 2001a, 2001b; Beta et al., 2000; Beta et al., 2001; Cruzy Celis et al., 1996; Hugo et al., 2000; McDonough et al., 1998; Moheno-Pérez et al., 1997; Suhendro et al., 2000; Taylor et al., 1997; Xie and Seib 2000). Previous studies reported RVA procedures with 13-min, 18-min, and 22-min temperature profiles. Beta et al. (1995) assayed α -amylase in sorghum malts by measuring the reduction in viscosity using a 3-min rapid test. To simulate an industrial mashing process, Goode et al. (2005) used RVA as a rheological tool to characterize the effect of the malt-to-barley adjunct ratio on viscosity and found clear correlations between the level of barley adjunct and the RVA parameters.

We hypothesized that sorghum cultivars with high starch contents are generally associated with higher RVA peak and final viscosities, which result from larger amounts of gelatinized substrates, and produce higher ethanol yields than cultivars with low starch contents. Tannins are well known for their effects on inhibition of α -amylase from porcine pancreas (Davis and Hosene, 1979), *Bacillus subtilis* (Reichert, 1980), and *Bacillus licheniformis* (Wu et al., 2007). Measurement of α -amylase activity with RVA described by the AACC Approved Method 22-08 (AACC International, 2000) is based on the ability of α -amylase to liquefy a starch gel. Thus, we anticipated that tannins in sorghum should be related to RVA parameters to some extent. To date, published literature contains no reports of using RVA to evaluate tannins in grain sorghum.

Many laboratory dry-grind procedures, all of which belong to the batch cooking system (Kelsall and Lyons 2003), have been developed. For most procedures, fermentation slurry with a first dose of α -amylase was cooked at 90-95°C for 45 min or 60 min (Ingledew et al., 1995; Ingledew et al., 1999; Thomas and Ingledew, 1990; Thomas et al., 1995; Wang et al., 1997, 1999; Zhan

et al., 2003; Wu et al., 2006a, 2006b, and 2007). After slurry temperature was reduced to 80°C, a second dose of α -amylase was added, and liquefaction proceeded for an additional 30 min. In some procedures, all the required α -amylase was added to the slurry in one step, and the slurry was cooked at 80°C for 60 min (Lee and Yoon, 2000), or at 85°C (Singh and Graeber, 2005) or 90°C (Singh et al., 2006) for 90 min prior to the subsequent or simultaneous saccharification step. We assume that a modified RVA procedure, with optimized temperature, time, solids level, and enzyme dosage, could be used to simulate the cooking and liquefaction steps in the dry-grind ethanol process and to quantitatively characterize the mashing properties of sorghum grains.

Therefore, the objectives of this study were: to characterize the pasting properties of sorghum grains; to simulate the cooking step in a laboratory dry-grind process and identify the mashing properties; to relate the RVA parameters to ethanol fermentation; and to optimize α -amylase dosage used for fuel ethanol production.

Materials & Methods

Sorghum Cultivars and Preparation

A population of 68 sorghum genotypes and elite hybrids, as described and used by Wu et al., (2007), were obtained from the 2004 winter breeding nursery of NC+ Hybrids (Monsanto subsidiary) in Puerto Rico. Tannins in the sorghum samples that had pigmented testas were deactivated using the formaldehyde method of Daiber and Taylor (1982) as follows: grain (100 g) of sorghum cultivars was steeped for 6 hr at room temperature in 100 mL of 0.04% (w/v) formaldehyde or distilled water. Grain was then blotted dry and dried further at 49°C for 16 hr. The water-steeped samples were used as controls. For some cultivars, whole kernels (500 g) were decorticated using a tangential abrasive dehulling device (TADD) equipped with an 80-grit abrasive pad (Venebles Machine Works Ltd, Saskatoon, Canada). The abrasive pad was shimmed to a minimum distance from the upper plate. The decortication level was controlled to about 20% (by weight) by adjusting the abrasive time. The original, steeped, and decorticated samples were ground using a mill (Udy Corp., Fort Collins, CO) through a 1.0-mm screen and used for chemical analysis and RVA testing. Samples for ethanol fermentation were ground into fine meals in a Magic Mill III Plus grain mill (Magic Mill Products & Appliances, Monsey, NY) set at level III.

RVA Viscosity Measurements

For model RVA-3D, a 23-min gelatinization, pasting, and setback profile, as described by the AACC Approved Method 76-21 (AACC International, 2000), was used. The actual profile is outlined in Table 1. Ground samples (4.00 g, 14% wb) were dispersed in 25.00 g of distilled water in aluminum canisters. The RVA parameters measured were pasting temperature, peak time, peak viscosity (the maximum hot paste viscosity), holding strength (the trough at the minimum hot paste viscosity), and final viscosity (the viscosity at the end of the test after cooling to 50°C and holding at this temperature). Breakdown was calculated by the difference between peak viscosity and holding strength, and setback was defined as the difference between final viscosity and holding strength.

A 10-min liquefaction test was carried out in model RVA-4 as described by Wu et al., (2007). The temperature profile was set to maintain a constant block temperature of 95°C for 10 min. An enzyme solution was prepared by diluting 2.30 mL of heat-stable α -amylase (Liquozyme SC DC from Novozymes) to 1 L distilled water. For most experiments, one mL of the enzyme solution containing 2.30 μ L of Liquozyme was added in a canister to liquefy the solids (8.00 g, 14% wb). The enzyme dosage was calculated based on 10 μ L of heat-stable α -amylase per 30 g of dry

solids in a normal fermentation test. In other experiments, the α -amylase levels in the slurries were multiples (may be greater or less than 1) of the normal dosage. The total weight of water and the required enzyme solution was kept constant at 21.00 g (14% wb). Peak viscosity, peak time, and final viscosity were measured.

Table 1. A Standard 23-min Gelatinization, Pasting, and Setback Profile

Stage	RVA STD2 Profile
Initial temperature	50°C
Initial holding time	1 min
Heating time from 50 to 95 °C	7.5 min at heating rate 6°C/min
Holding temperature	95°C for 5 min
Cooling time from 95 to 50 °C	7.5 min at cooling rate 6°C/min
Final temperature	50°C
Final holding time	2 min
Total test time	23 min

Before initiating a sample measurement, a plastic paddle was attached to the stirring head of the RVA and zeroed at 160 rpm against air (Goode et al., 2005). After a sample was poured into the water, a plastic paddle was inserted into the sample canister, rotated, and jogged up and down by hand for 15-30 sec to remove lumps. For all RVA measurements, the samples were premixed for 10 sec at 960 rpm, whereafter a speed of 160 rpm was applied. Rheological measurement data were recorded at 4-sec intervals and stored by RVA dedicated software.

Analytical methods

Moisture content was measured using the AACC Approved Method 44-15A (AACC International, 2000). Total starch content was determined using Megazyme total starch kits according to the AACC Approved Method 76-13 (AACC International, 2000). Amylose content of starch was analyzed by the method of Gibson et al., (1997) using an amylose-amylopectin assay kit (Megazyme). Tannin content was evaluated using the modified vanillin/HCl assay of Price et al., (1978) with catechin as the standard. Ethanol fermentation was the same procedure as described by Wu et al., (2006b).

Statistical Analysis

All experiments were performed at least in duplicate. The tabular results quoted are the mean values of the repeated experiments. The viscosity curves shown represent one sample measurements. Analysis of variance (ANOVA), least significant difference (LSD), split-plot design, and linear regression were performed using SAS software version 9.1 (SAS institute, Cary, NC).

Results and Discussion

Pasting Properties of Ground Sorghum Grains

The 23-min standard temperature profile was applied to measure the pasting properties of 68 ground sorghum grains at a solids level of 11.86% (w/w). The numerical data generated by RVA are summarized in Table 2. Typical pasting curves, which were selected to represent the 68 sorghum cultivars based on their peak viscosities, are displayed in Fig. 1.

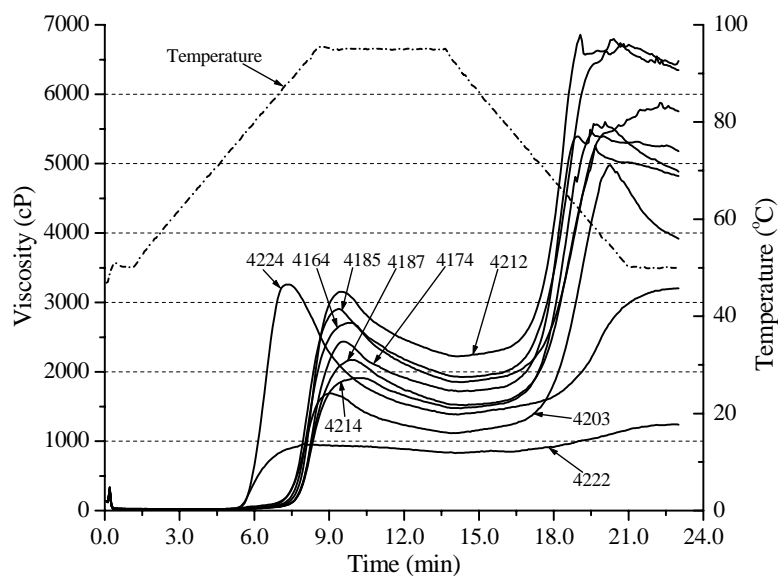


Figure 1. Pasting curves of 9 sorghum samples selected from 68 cultivars and measured using the 23-min temperature profile in model RVA-3D.

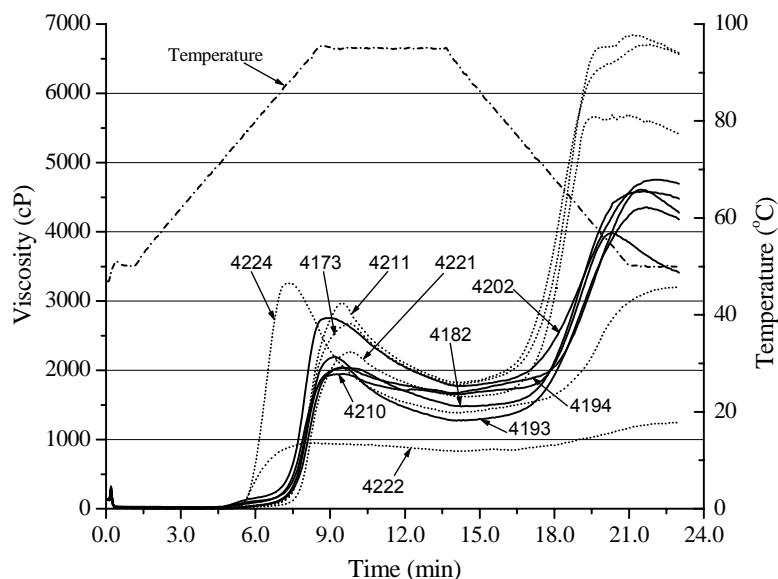


Figure 2. Pasting curves of 10 sorghum samples selected from 68 cultivars and measured using the 23-min temperature profile in model RVA-3D. Samples with a slow liquefaction speed are 4182, 4193, 4194, 4202, and 4210 (solid lines); samples with a quick liquefaction speed are 4173, 4211, 4221, 4222, and 4224 (dotted lines).

The coefficients of determination (R^2) for RVA parameters and total starch, ethanol yield, and conversion efficiency are summarized in [Table 3](#). Total starch was highly correlated with final viscosity as well as setback ($R^2 = 0.60$, $p < 0.0001$ for final viscosity and $R^2 = 0.55$, $p < 0.0001$

for setback, respectively). The relationships between ethanol yield and RVA parameters such as peak viscosity, holding strength, breakdown, final viscosity, and setback give strong support to the hypothesis that pasting properties of sorghum can be related to ethanol fermentation.

Representative RVA curves of samples with distinctive liquefaction speeds are shown in Fig. 2. Except for the two waxy samples 4222 and 4224, it is clear that there were no differences in peak viscosity, holding strength, and breakdown between the two group samples with different liquefaction characteristics. For example, sample 4211 was easily liquefied but had a higher peak viscosity, while sample 4182 was easily agglomerated but had a lower peak viscosity. Therefore, viscosities measured using the 23-min temperature profile could not be used to explain the difference in mashing characteristics among the sorghum samples.

Table 2. RVA Test Results for Sorghum Samples Using the 23-min Temperature Profile in Model RVA-3D

Parameters	Peak Viscosity (cP)	Holding Strength (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (min)	Pasting Temperature (°C)
Minimum	911	785	126	1193	408	7.3	76.7
Maximum	3213	2177	1874	7308	5443	10.3	89.6
Average	2352	1615	737	5088	3473	9.5	87.1
Standard Error	36	21	23	35	39	0.067	0.32

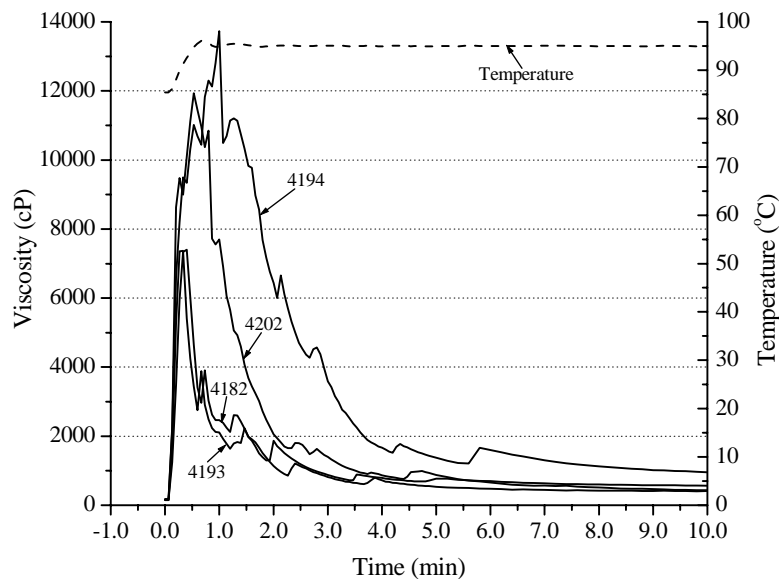


Figure 3-1. Viscosity curves of 4 sorghum samples with peak higher than 7000 cP, measured using the 10-min temperature profile in model RVA-4 with the normal dosage of α -amylase.

Mashing Properties of Ground Sorghum Grains

As shown in Fig. 3, starch in the slurry gelatinized almost immediately when the canisters were put into a block that had been pre-heated to 95°C, and viscosity of the slurries increased dramatically. Meanwhile, the heat-stable α -amylase tended to reduce viscosity by liquefying the

gelatinized starch. There was a balance between gelatinization and liquefaction, which led to peak viscosity. When gelatinization dominated, viscosity increased until reaching the peak value. Viscosity decreased gradually after peaks with the slurries stirred continuously and the block temperature maintained at a constant of 95°C.

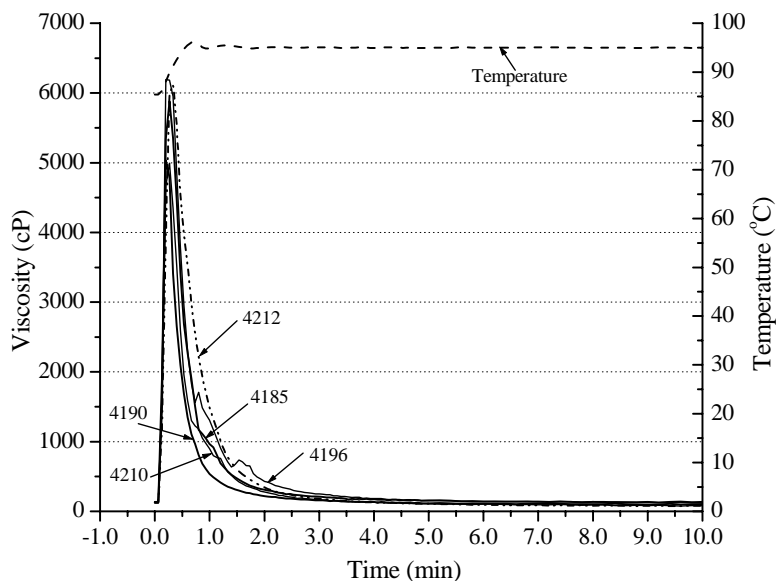


Figure 3-2. Viscosity curves of 5 sorghum samples with peak from 4000 to 7000 cP, measured using the 10-min temperature profile in model RVA-4 with the normal dosage of α -amylase.

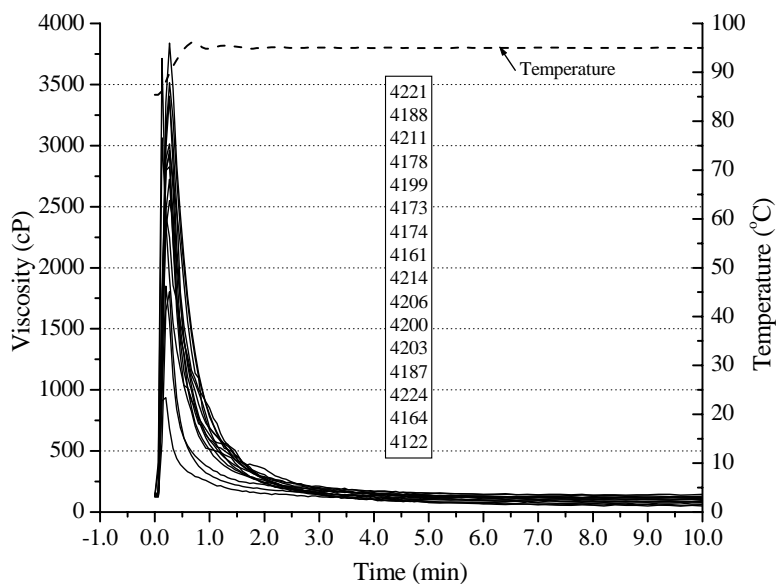


Figure 3-3. Viscosity curves of 16 sorghum samples with peak less than 4000 cP, measured using the 10-min temperature profile in model RVA-4 with the normal dosage of α -amylase. Codes are listed from top to bottom according to peak viscosity, starting with the highest value.

The 25 samples were divided into 3 groups (Figs. 3-1, 3-2, and 3-3) according to their peak viscosities. The samples with a slow liquefaction rate in Fig. 2 were classified into group one (Fig. 3-1) and group two (Fig. 3-2). All samples with tannins except for 4188 and 4199 belonged to these two groups, suggesting that tannins could be an important factor affecting mashing properties. The samples with a quick liquefaction rate in Fig. 2 were placed in the third group (Fig. 3-3).

The difference in mashing properties among sorghum grains (Fig. 3) with a normal dosage of exogenous enzyme suggested that the α -amylase in some samples had been inhibited by some substance, perhaps tannins in those sorghums that contained a pigmented testa (Davis and Hosney 1979; Reichert 1980).

Table 3. Coefficient of Determination (R^2) for RVA Parameters and Total Starch, Ethanol Yield, and Conversion Efficiency ^a

	RVA Parameters						
	Peak Viscosity	Holding Strength	Breakdown	Final Viscosity	Setback	Peak Time	Pasting Temperature
Total Starch	0.44***	0.29***	0.30***	0.60***	0.55***	0.0019 ^{NS}	0.0060 ^{NS}
Ethanol Yield	0.47***	0.27***	0.36***	0.61***	0.57***	0.0017 ^{NS}	0.0043 ^{NS}
Conversion Efficiency	0.16***	0.05 ^{NS}	0.17***	0.18***	0.18***	0.0003 ^{NS}	0.0005 ^{NS}

^a RVA parameters were measured using the 23-min temperature profile in model RVA-3D.

^{NS} Not Significant at 5% level; *** significant at 0.1% level

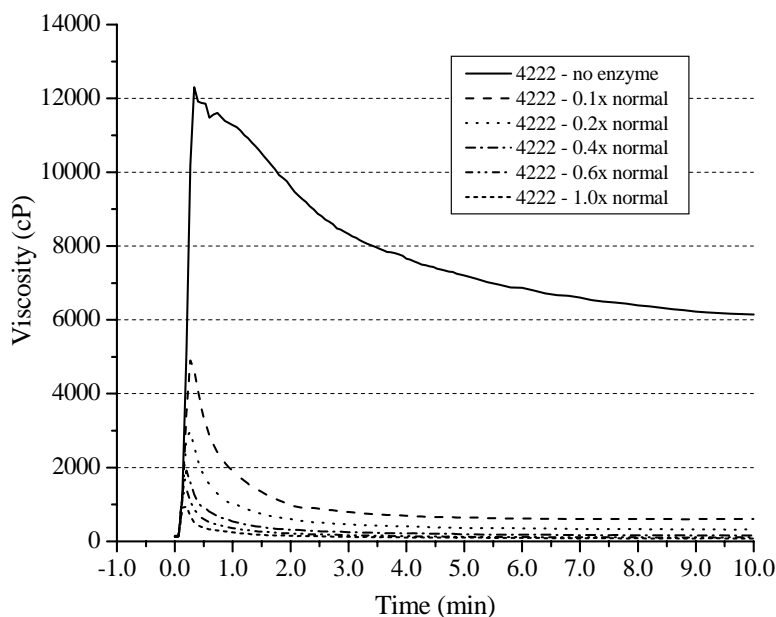


Figure 4. Viscosity curves of sample 4222 measured using the 10-min temperature profile in model RVA-4 with increased levels of α -amylase in the slurries. The normal dosage of α -amylase contained 2.30 μ L of Liquozyme for liquefying the solids (8.00 g, 14% wb) in a canister.

Effect of Thermostable Alpha-Amylase on Mashing Properties

Fig. 4 shows the powerful liquefying action of α -amylase, which lowered viscosity sharply even at very low activity (e.g., 0.1x normal dosage). All RVA parameters (peak viscosity, peak time, final viscosity, rate of viscosity breakdown after peak, and area under curve) decreased remarkably with increasing levels of heat-stable α -amylase in the slurries.

Alpha-amylase was intensively inhibited in the mashes of sample 4194 (Fig. 5). With increasing enzyme activity, peak time and final viscosity decreased slightly. However, peak viscosity did not change significantly ($p = 0.138$), even when the enzyme level was 20 times the normal dosage. Sample 4194 was the most difficult to liquefy during mashing in the fermentation tests. Without additional and careful shaking, it was very difficult to disperse and completely liquefy gelled particles. The inactivation and removal of tannins resulted in significant reduction in peak and final viscosities of sorghum grains (Fig. 5). After such treatments, the sorghum grains with tannins had similar peak viscosities to non-tannin grains shown in Fig. 3.

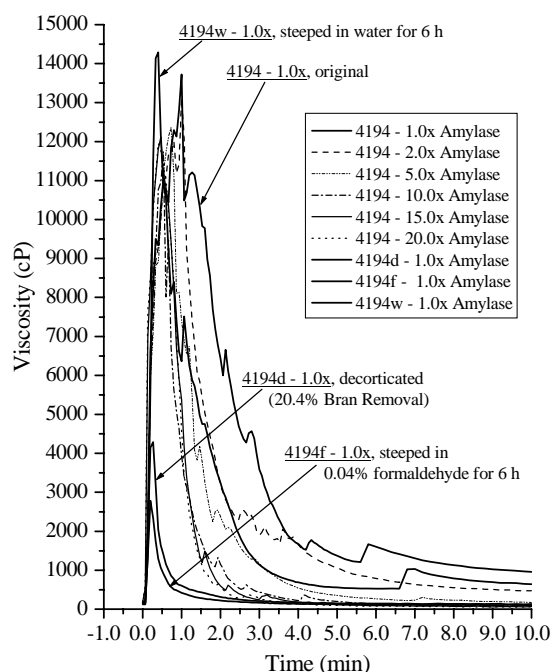


Figure 5. Viscosity curves of sample 4194 measured using the 10-min temperature profile in model RVA-4 with increased levels of α -amylase in the slurries. The normal dosage of α -amylase contained 2.30 μ L of Liquozyme for liquefying the solids (8.00 g, 14% wb) in a canister.

Effect of Tannins on Mashing Properties

For the 9 samples with tannins (Fig. 6), there was a strong linear relationship between tannin content and final viscosity ($R^2 = 0.91$, $p < 0.0001$) and peak viscosity ($R^2 = 0.89$, $p = 0.0001$). In addition, peak time was also highly correlated to tannin content ($R^2 = 0.89$, $p = 0.0001$). These results indicate that RVA could be used to quickly predict tannin contents in sorghum grains.

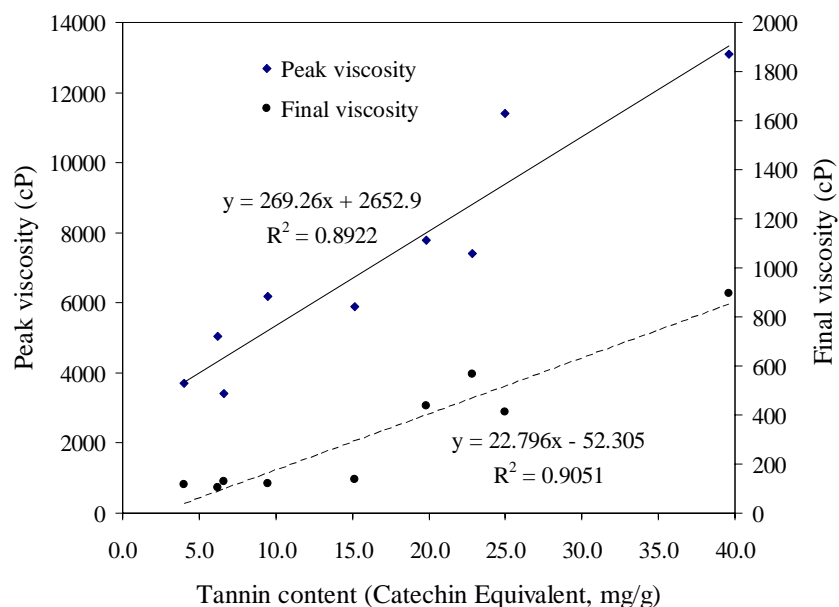


Figure 6. Relationships between tannin content and peak and final viscosity measured using the 10-min temperature profile with the normal dosage of α -amylase.

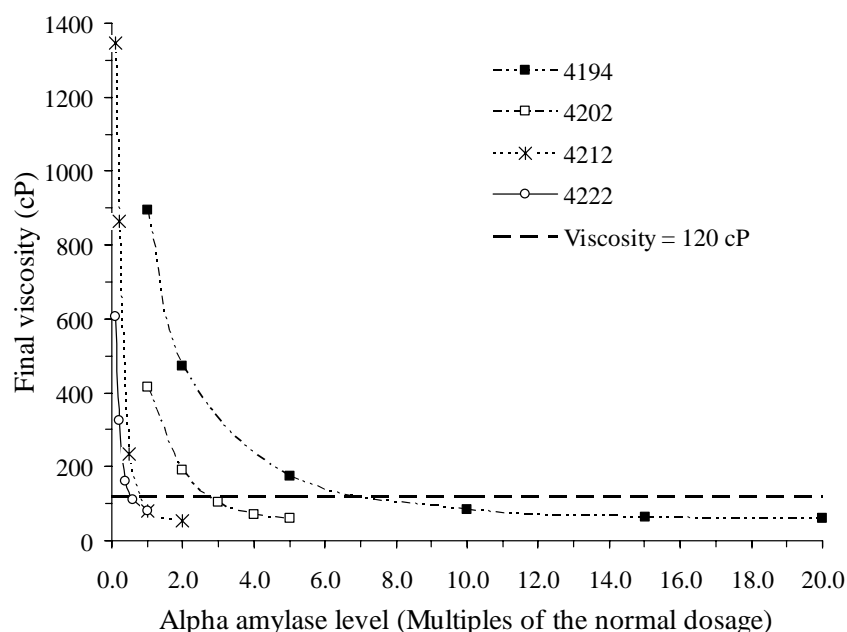


Figure 7. Effects of α -amylase levels on final viscosities of different sorghum grains measured using the 10-min temperature profile in model RVA-4. The normal dosage of α -amylase contained 2.30 μ L of Liquozyme SC DC for liquefying the solids (8.00 g, 14% wb) in a canister.

Optimization of Alpha-Amylase Doses for Mashing

Relationships between final viscosities and levels of α -amylase for representative grains are displayed in Fig. 7. Enzyme levels were expressed as multiples of the normal dosage. When the best-fit curves were applied to the data, clear power correlations were found between final

viscosity and α -amylase level ($R^2 = 0.991, 0.995, 0.995, \text{ and } 0.981$, for samples 4194, 4202, 4212, and 4222, respectively with $p < 0.0001$). Different grains fitted different power curves, and most grains behaved like samples 4212 and 4222. The optimized enzyme doses to obtain less than 120 cP of final viscosities varied among grains. The 4 samples in Fig. 3-1 required more than twice the normal dosage of α -amylase, while 9 samples in Fig. 3-3 needed only 50-80% of the normal level. As shown in Fig. 7, final viscosities could be reduced to a value of $\approx 40\text{-}45$ cP, which was the viscosity of a slurry at 60°C before starch gelatinization (data not shown), even with higher enzyme levels.

Conclusion

The feasibility of using RVA as a tool for assessing the quality of grain sorghum to produce fuel ethanol was investigated in this study. For the 23-min gelatinization, pasting, and setback profile, there was a strong linear relationship between ethanol yield and final viscosity as well as setback. From this point, RVA could be used as a tool to predict ethanol yield. Sorghum cultivars with higher peak and final viscosities, resulting from larger amounts of gelatinized substrates, will produce higher ethanol yields than those with low viscosities. The differences in mashing properties among sorghum grains were enlarged and quantified using the 10-min liquefaction test. There was a remarkable difference in mashing properties among representative grains with the normal dosage of α -amylase. It will be very helpful for producers to quickly screen out the grains with abnormal high peak viscosities using RVA. Tannin content was found to be highly correlated to mashing properties. The 10-min RVA procedure could be used as a quick method to predict tannin levels in sorghum grains. For all grains, final viscosities decreased remarkably with increasing levels of heat-stable α -amylase in the slurries. Clear power correlations were found between final viscosities and α -amylase levels. Different grains fitted different power curves, and the optimized enzyme doses to obtain less than 120 cP of final viscosities varied greatly among grains. These results showed that RVA could be used as a tool to optimize α -amylase doses used for ethanol fuel production. Moreover, RVA could be used for assessment of different commercial enzyme preparations.

Acknowledgments

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